13. Drug Discovery and Development

13.001 Screening for carcinoma cell lines confirmed a hit in drug discovery. Antunes JE[1], Pereira MBM[2], Ribeiro RT[1] [1]UFJF – Farmácia, [2]UFJF – Ciências Básicas em Saúde

Introduction: The development of pharmacological agents with specific function has been used in several important aspects of research in biology, and to establish new therapeutic strategies for diseases[1]. The discovery of new drugs is a long challenge that requires continuous and substantial financial investment in research. In this context, computational studies can aid the discovery of new drugs. Studies of pharmacokinetic parameters, *molecular docking*, prior *in vitro* can to be used to select new drugs[2]. The screening of molecules is an important step in drug discovery. Major diseases such as cancer may present new drugs for treatment after these screening[3]. **Aims**: Drug Discovery by computational methods; screening to select the most effective compound to inhibit carcinoma cell lines. **Methods**: We use the main method used in drug discovery: Hit to lead in drug design and in drug discovery. It has been identified and validated a biological target, using computational studies including molecular docking and pharmacokinetic studies to select the hits. The hits were confirmed by primary assay such as MTT assay. The MTT assay is a colorimetric assay for assessing cell viability. The screening test was performed with 22 compounds and differents cell lines of solid tumor. The result was compared to doxorubicin used as reference drug. The compounds used in this work are quinazolines. It were characterized by NMR and mass spectrometer[5]. The compound **D5** is named N- [4- (Benzyloxy) phenyl] -6,7-dimethoxyquinazolin-4-amine. **Results and Discussion**: We work to develop new drugs by computational methods. The hits were evaluated by MTT assay in screening to select the most effective compounds to inhibit carcinoma cell lines. The results showed that a compound called **D5** was efficient to inhibit carcinomas lines. This result was very interesting because **D5** was better than doxorubicin. **D5** was able to inhibit various cell lines including U87 (human astrocytoma cells) carcinoma. These cells were inhibited by approximately 95% while doxorubicin inhibited approximately 80%. **D5** is a compound promising because there are few drugs to treatment for astrocytoma cancer[5]. **Conclusions**: This work allowed select a new compound. Computational tools such as pharmacokinetic parameters and *docking* studies were used to select more promising molecules. After synthesis, screening for carcinoma cell lines has confirmed the **D5** compound as a hit, but other tests will be need to confirm it as a leader. **Acknowledgements**: Our acknowledgements are to Department of Pharmacy, Federal University of Juiz de Fora, Governador Valadares, Brazil. **References**: 1. Golan, D. E.; Tashjian, A. H.; Jr.; Armstrong, E. J. April W. Armstrong, A. W. Principleof Pharmacology, 2a ed. 2009, chapter 1. 2. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Adv. Drug Deliv. Rev. 1997,23: 3-25. 3. Ozkal, S.; Paterson, J.C. Tedoldi, S.; Hansmann, M.L.; Kargi, A.; Manek, S.; Mason, D.Y.; Marafioti, T. Pathol Res Pract. 2009, 11: 781-88. 4. http: //www.bibliotecadigital.unicamp.br/document/?code=000902133&opt=4 - Tese: Novas quinazolinas 2,4,8-dissubstituídas com potencial atividade de inibição da quinase de adesão focal (FAK), 2013. 5. Giannone, G.; Rondé, P.; Gaire, M.; Haiech, J.; Takeda, K. J. Biol. Chem. 2002, 277: 26364–26371.

13.002 Protective effects of green tea against Leukemic Immune Suppression. Calgarotto AK[1], Pericole FV[1], Maso V[1], Longhini AL[1], Favaro P[2], Santo IP[1], Duarte ASS[1], Saad ATO[1] [1]FCM-Unicamp, [2]Unifesp-Diadema

Introduction: The beneficial effects of green tea (GT) are well known. A number of epidemiologic studies have linked consumption of green tea to decreased risk of cancer, *in vitro* and animal models have supported green tea's ability to inhibit the different stages of cancer development. The major hypothesis explaining such effects is that the high levels of polyphenolic compounds in tea can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals (ROS). Accumulation of ROS triggers oxidative stress in various cell types and contributes to the development, progression, and persistence of cancer. Recent research has demonstrated that redox dysregulation caused by ROS promotes proliferation, differentiation, genomic, and epigenetic alterations; immune evasion; and survival in leukemic cells. Acute myeloid leukemia (AML) is a malignant hematopoietic disorder characterized by proliferation of immature myeloid precursors with impairment of the immune system. **Aims**: Our primary goal was to confirm the antioxidant activity of GT in leukemic blasts of AML patients. Our secondary objective was to analyze possible changes in their T cells signaling induced by GT. **Methods**: We are performing a study with elderly AML patients unfit to conventional chemotherapy. The patients received 1 capsule containing 500 mg of GT once per day during 6 months and they were evaluated once every 4 weeks by physical examination and laboratory testing. **Results and Conclusions**: Treatment with GT induced a drastic reduction of ROS accumulation in blast cells of patient's bone marrow. We could also observe an increase in the percentage of CD3+CD8+ T cells with decrease of regulatory T cells (T-reg) which may suppress the immune response against cancer. Human Tregs traffic to and are retained in bone marrow through CXCR4/SDF1 signals. Our results show a significantly reduction on the CXCR4 receptor percentage in Treg as well as in CD34+ cells after GT treatment. Leukemic cells may increase ROS generation and expression of survival genes, like CXCR4, to escape oxidative stress and avoid death, then, in this scenario our results pointing the GT antioxidant activity can also be related to the ability to modulate CXCR4/ SDF1 signaling. **Financial Support**: Fundação de Amparo à Pesquisa de São Paulo. Research approval by the Human Research Ethical Committee: 14290713.8.0000.5404

13.003 Layered double hydroxides with intercalated indomethacin: Antinociceptive study and gastroprotective effect. Bentes-Lima A[1], Dias DRC[1], Queiroz-Santos GC[2], França CM[1], Anicete-Santos M[3], Nascimento JLM[3], Bastos GNT[2]
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Layered double hydroxides (LDHs) compound of magnesium and aluminium cations with indomethacin anti-inflammatory intercaled has been successfully prepared by coprecipitation method. Usually, these materials are applied to controlled release of drugs. The aim of this study was to assess the cytotoxicity of LDHs in blood cells, to evaluate the biodisponibility of indomethacin (IND) incorporated into LDHs (IND-LDHs) using models of nociception and to investigate the possible anti-ulcerogenic induced by indomethacin. The indomethacin was adsorbed to calcined material resulting in reduction of specific surface area, volume and pore diameter. For the *in vivo* test we used male albine Swiss mice (6-8 weeks). The experimental protocols were approved by the Ethical Committee for Research with Experimental Animals (CEPAE) of Universidade Federal do Pará (CEPAE-UFPA: 124-13). It was evaluated the hemolytic effect of LDHs. The erythrocytes were isolated and purifed by three successive washes with saline. The cells were incubated with LDHs at 250µg/ml. Controls were prepared in the same manner as the above erythrocytes samples except adding Triton-X (positive control) and DMSO (negative control) instead of the LDHs. After 1 h incubation at room temperature, the samples were spun down for the detection of hemoglobin released from hemolyzed erythrocytes. The results showed no hemolytic activity using our LDHs. IND-LDHs, IND, LDHs or vehicle were administered by gavage at 5 mg/kg. The drugs were administered 2, 24 or 48 hours before the i.p. injection of 0,6% acetic acid. The IND-LDHs decreased the number of writhing in 97.2%, 87.66% and 61.33%, to 2, 24 or 48 hours respectively. While the IND decreased the number of writhing only in 2 hours. Pharmacological studies *in vivo* show that intercalation of the drug in the LDH reduces the ulcerating damage of the drug. The results of *in vivo* drug release revealed that the IND-LDHs compound is a perspectival material for potential application as controlled drug delivery systems due it keeps the antinociceptive effect up to 48 hours with just one dose. **Acknowledgements:** The authors are grateful to Ministry of Science and Technology (MCT/CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Federal do Pará Brazilian Government, for financial support.

13.004 Layered double hydroxides intercalated with Norfloxacin: characterization X-ray diffractometry and hemolysis assay. França CM[1], Lima AB[1], Costa KM[1], Dias DRC[1], Remedios CRM[2], Anicete-Santos M[2], Alves CN[2] – [1]UFPA – Biotecnologia, [2]UFPA

Introduction: The layered double hydroxides (LDHs) structure consists of brucite-like [M(OH)2] sheets containing both bivalent and trivalent cations, resulting in positively charged sheets compensated by metal anions into the interlayer galleries. Usually, these materials are applied to control the release of drugs. **Aims**: In this work, the constant pH coprecipitation method was used to replace the nitrate anions of LDH with norfloxacin and all samples were characterised by X-ray diffractometry. Furthermore, we evaluated the cytotoxicity of LDHs in blood cells. **Methods**: The X-ray powder diffraction (XRPD) patterns were obtained on a BRUKER X-ray diffractometer D8-Advance using a copper source and one monochromator for selecting the Kα1 gratife emission region of copper, corresponding to a wavelength of 1.5406 Å. The potential difference of the source was 40kV and 40mA current. The standards will be obtained with step speed equal to the step 0.02º in the range of values (2θ) from 0 to 80. For the *in vitro* test we used one male albine Swiss mice (8 weeks). The experimental protocols were approved by the Ethical Committee for Research with Experimental Animals (CEPAE) of Universidade Federal do Pará (CEPAE-UFPA 123-15). With the hemolysis tests it was evaluated the hemolytic effect of LDHs. Under ketamine/xylazine anesthesia (i.p.). Briefly, mice blood was obtained from the marginal ear vein. After the erythrocytes were isolated and purified by three successive washes with saline. The cells were incubated with LDHs at 250µg/ml. Controls were prepared in the same manner as the above erythrocytes samples except adding Triton-X (positive control) and DMSO (negative control) instead of the LDHs. After 1 h incubation at room temperature, the samples were spun down for the detection of hemoglobin released from hemolyzed erythrocytes. The concentration of hemoglobin released from the hemolyzed RBCs was determined by measuring the absorbance of the supernatant at 541nm by UV/Vis spectroscopy. **Results**: The result of XRD for LDHs pure presents diffraction peak with basal spacing d003 = 7.8. Since LDH-NORF sample had values d003 = 13.2, and calculated according to the Bragg equation with values, respectively, 0.16 and 0.21, reflecting phases in well crystallized and ordered layers. LDH-NORF (66 nm) and DMSO (179 nm) should not be hemolytic. However, Triton-X should be hemolytic (475 nm) **Conclusions**: The increase in basal spacing indicates that norfloxacin has been successfully intercalated into interlayers of LDHs. The results showed no hemolytic activity using our LDHs. The results presented here provide a better understanding of LDHs interaction. **Acknowledgements:** The authors are grateful to Ministry of Science and Technology (MCT/CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Federal do Pará Brazilian Government, for financial support.

13.005 Development, characterization and evaluation of naringin and naringenin nanocapsules-induced cytotoxicity. Ferreira CF[1], Cordenonsi LM[2], Sulczewski FB[3], Liszbinski RB[3], Rodrigues LJ[1], Boeck CR[1], Raffin RP[1] [1]Unifra – Nanociências, [2]UFRGS – Ciências Farmacêuticas, [3]Unifra – Biomedicina

Introduction: Several evidences indicate that reactive species (RS) and oxidative stress contribute to the pathophysiology of dementia. An innovative alternative to protection the brain is the use of flavonoids with antioxidant effect and brain activity in dementia conditions such as naringin and naringenin. However, these two compounds exhibit low bioavailability when administered orally. An alternative to overcome this limitation would be to use nanocarriers, such as nanocapsules (NCS), which allow increasing its bioavailability and effectiveness. **Aim of the study**: Thus, this study aims to development and characterization of nanocapsules containing naringin and naringenin. Furthermore, it was found possible effects on cell viability in Vero cell line after treatment with nanoencapsulated drugs through the technique based on the metabolism of the reagent 3- (4,5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide) (MTT). For this porpouse, the production of previous developed polymeric nanocapsules and this physical and chemical parameters determined. This naringin and naringenin nanocapsule suspensions were prepared at the concentration of 2 mg/mL using interfacial deposition of pre-formed polymer technique containing as polymer Eudragit® L100. **Results**: Naringin and naringenin nanocapsules showed acid pH, particle diameter lower than 95 nm, polydispersity index lower than 0.2, zeta potential of -12,46 to -17,6 mV. The MTT assay results show that the suspensions NCS caused acute toxic effects observed by a significant reduction in Vero cells viability for periods of 24 and 72 hours of incubation, at concentrations of 5, 50 and 500µg/mL compared to the control. Conclusions: The nanoencapsulation of the flavonoids proved feasible by using Eudragit® L 100 as a polymer, presenting a homogeneous particle size, acid pH, negative zeta potential and adequate encapsulation rate and showing characteristics suitable for oral administration. Furthermore, thus one can conclude that the concentration of low cytotoxicity to the naringin and naringenin less than 5 µg/mL. **Acknowledgements**: Research supported by FAPERGS, CAPES and CNPQ.

13.006 Gastro-protective and anti-edematogenic effects of ibuprofen intercalated in layered double hydroxide carrier. Bentes Lima A[1], Queiroz Santos GC[2], França CM[1], Anicete-Santos M[1], Nascimento JLM[3], Bastos GNT[3] – [1]UFPA – Biotecnologia, [2]UFPA – Biologia Celular e Molecular, [3]UFPA – Ciências Biológicas

Layered double hydroxides (LDHs) compound of magnesium and aluminium cations with ibuprofen anti-inflammatory intercaled has been successfully prepared by coprecipitation method. Usually, these materials are applied to controlled release of drugs. The aim of this study was to evaluate the biodisponibility of ibuprofen incorporated into LDHs (IBU-LDHs) using models of acute inflammation induced with carrageenan and to investigate the possible anti-ulcerogenic induced by ibuprofen. The ibuprofen was adsorbed to calcined material resulting in reduction of specific surface area, volume and pore diameter. Anti-inflammatory effects of IBU-LDHs have been investigated on 4 rat groups with carrageenan (CAR)-induced paw oedema model. IBU-LDHs, LDHs or vehicle, were administered by gavage at 200mg/kg. The drugs were administered 2, 24, 48 or 72 hours before the i.p. injection of carrageenan. The experimental protocols were approved by the Ethical Committee for Research with Experimental Animals (CEPAE) of Universidade Federal do Pará (CEPAE-UFPA: 124-13). Carrageenan-induced paw oedema was significantly reduced by treatment with IBU-LDHs at 2nd, 3rd and 4th h after injection of carrageenan, to 2, 24, 48, or 72 h respectively. Pharmacological studies *in vivo* show that intercalation of the drug in the LDH reduces the ulcerating damage of the drug. The results of *in vivo* drug release revealed that the IBU-LDHs compound is a prominent material for potential application as controlled drug delivery systems due it keeps the anti-inflammatory effect up to 72 hours with just one dose. **Acknowledgements:** The authors are grateful to Ministry of Science and Technology (MCT/CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Universidade Federal do Pará Brazilian Government, for financial support.

13.007 Novel partial agonist of PPAR-gamma (LASSBio-1773) reduces neuropathic pain in diabetic rats. Araujo JSC, Dias JL, de Silva JS, Trachez MM, Delgobbo MS, Silva TF, Lima LM, Barreiro EJ, Sudo RT, Zapata-Sudo G UFRJ – Farmacologia e Química Medicinal

Introduction: Diabetes mellitus (DM) is a multifactorial disorder characterized by high blood glucose levels, which is associated to a neuropathic pain (DNP), one of the comorbidities associated with DM. Agonists of the peroxisome proliferator-activated receptor gamma (PPAR-gamma) is an antidiabetic drug which recently, have been showing beneficial effects in experimental model of DNP. **Aims**: This work aims to evaluate the effects of a novel characterized partial agonist of the PPAR-gamma (LASSBio-1773) in a diabetes-induced chronic pain model in rats. **Methods**: Protocols were approved by Animal Care and Use Committee at Universidade Federal do Rio de Janeiro (DFBCICB060). Diabetes was induced by a single intravenous injection of streptozotocin (STZ, 60 mg/kg) in male Wistar rats (200 - 240 g). DNP was confirmed after observation of the mechanical allodynia and thermal hyperalgesia. Both parameters were evaluated using the paw withdrawal threshold (PWT) and thermal heat withdrawal latency (TWL) in diabetic rats. Eight weeks after STZ injection and DNP establishment, rats were randomly divided into 2 groups (8 animals per group) and treated with either vehicle (dimethylsulfoxide, DMSO) or LASSBio-1773 (50 mg/kg/day, i.p.) for 7 days. **Results**: Eight weeks after STZ injection, blood glucose levels increased from 97,4 ± 4,7 to 337,1 ± 39,5 mg/dL ($P < 0.001$) and the neuropathic pain symptoms were detected in those rats. PWT (g) was 50.6 ± 0.7 before STZ injection (baseline) and it reduced to 27.8 ± 0.6 ($P < 0.05$) after 8 weeks. LASSBio-1773 recovered PWT to 43.7 ± 1.1 g ($P < 0.05$) and to 47.5 ± 1.2 g ($P < 0.001$) after 3 and 7 days of treatment. TWL (s) was 24.4 ± 1.3 before diabetic condition and it reduced to 9.4 ± 0.4 after 8 weeks of DM. LASSBio-1773 increased TWL to 15.6 ± 0.7 ($P < 0.001$) after 7 days of treatment, indicating that LASSBio-1773 decreased the neuropathic pain induced by diabetes. **Conclusion**: The new compound LASSBio-1773, a partial agonist of the PPAR-gamma, is a promising therapeutic candidate for the treatment of DNP, because it reduced thermal hyperalgesia and mechanical allodynia in diabetic rats. **Financial support**: CNPq, CAPES, FAPERJ, INCT-INOFAR, PRONEX, PENSA RIO

13.008 Effect of chronic treatment with creatine nanoliposomes on hepatic and hematologic toxicity parameters in rats. Moreira MP[1], Borin DB[1], Mezzomo NJ[1], Biacchi K[2], Amaral RG[2], Rech VC[1], Boeck CR[1] – [1]Unifra – Nanociências, [2]Unifra – Acadêmico

Introduction: Liposomes are lamellar structures made of one or more phospholipid bilayers surrounding aqueous or lipid core. Several methods describe preparation of liposomes, including the ethanol injection method, normally used to obtain monolayer liposomes. Liposomes are biocompatible and biodegradable nanoparticles, however these particles may become toxic with the addition of synthetic phospholipid or polymer in their surface. Creatine is important supply energy to all cells in the body synthesized in the liver, pancreas, kidney and brain; but also it can be obtained by ingestion of red meat and fish. Creatine kinase converts creatine to phosphocreatine contributing to the energetic homeostasis of tissues. Studies have attributed cardioprotective and neuroprotective effects to the creatine. To improve its low permeability across blood brain barrier, creatine liposomes would be a strategy to deliver the drug to the brain. **Aims**: Evaluate hepatic and hematologic toxicity parameters in young rats pretreated chronically with creatine liposomes. **Methods**: Creatine liposomes were prepared as described by Justo and Moraes (2005), with slight modification. Phospholipid, cholesterol, PE-PEG 2000 and Tween 80 (70: 20: 5: 5, respectively) were solubilized in ethanol and injected in PBS, pH 7.4 with creatine (10 mg/mL) with slow homogenization. The organic phase was removed to the liposomal system. Creatine liposomes obtained were applied to a microfluidizer and the physicochemical characterizations was performed. Male Wistar rats were treated from seventh to 19th day with free form or liposomes of creatine at dose of 0.4 mg/g, followed by toxicity analysis. **Results**: Particle size was 215.4 ± 3 nm, zeta potential was -123 ± 1 mV and PDI was 0.240. Hepatic and hematological markers were not altered by creatine liposomes treatment in young rats. **Conclusion**: Creatine liposomes showed optimum physicochemical proprieties and they did not induce any alteration in red or white series of blood, demonstrating to be saving for hematopoietic system. In the same way, creatine liposomes did not induce any alterations on hepatic parameters. Thus, creatine nanoliposomes can be applied in different models of animals including neurodegenerative diseases. Reference: JUSTO, O.R. J. Pharmacol Pharmacother, v.57. p.23.2005. Financial research support: FAPERGS, CAPES. All animal procedures were approved by the Animal Ethics Committee from the Centro Universitário Franciscano (protocol number: 006/2014).

13.009 LFQM 75: New lead compound for Alzheimer's disease treatment. Souza INO[1], Pereira TS[1], Boni MS[1], da Silva FMR[1], Viegas Jr C[2], Castro NG[1], Neves G[1] [1]ICB-UFRJ, [2]Unifal

Introduction: Alzheimer's disease (AD) is a neurodegenerative condition characterized by progressive cognitive decline that affects recent memory in early stages, evolving to a severe compromise of memory and cognition. It is considered the most common type of dementia and affects about 50-60% of the world elderly population. Because it's a disabling disease, it presents strong socioeconomic consequences. Acetylcholinesterase inhibitors (such as donepezil) enhance the central cholinergic function therefore being the most used pharmacological strategy for minimizing AD symptoms. However, since they don't have central selectivity, these drugs have considerable side effects making it difficult to scale up the dosage or to achieve appropriate drug compliance. Therefore, the search for new, more selective and favorable substances for AD treatment is justified. With such objective in mind, a series of innovative compounds with potential acetylcholinesterase inhibitory activity was planned through hybridization of donepezil's pharmacofore and the phenyl-N-acylhydrazone moiety, a privileged scaffold with reported anti-inflammatory activity. This rational approach aimed to design new compounds able to simultaneously induce the symptomatic relief of cholinergic therapy and suppress the chronic neuroinflammation present in AD, possibly modifying the neurodegenerative process. Seventeen novel compounds were synthesized and pharmacologically evaluated, among which LFQM 75 stood out. **Aims**: The main objective of this work was to further characterize the cholinergic actions of LFQM 75 using *in vitro* and in vivo pharmacological assays. **Methods**: First, LFQM 75 cholinesterase inhibitory activity was assessed *in vitro* using the Ellman's assay. Then, its ability to block scopolamine-induced cholinergic amnesia (0.3 mg/kg i.p.) in the novel object recognition paradigm was investigated. Adult male Swiss mice (CECAL/Fiocruz) were used. LFQM 75 was administered at 100 mmol/kg per os. **Results**: LFQM 75 presented a half maximal inhibitory concentration (IC50) of 10.89 μM for acetylcholinesterase and 58.14 μM for butyrylcholinesterase. It was characterized as a non-competitive inhibitor of acetylcholinesterase. In mice, LFQM 75 was able to block the cholinergic amnesia in the novel object recognition paradigm (62.0 ± 3.4 % of time exploring the novel object in contrast with 47.2 ± 1.9 % by the scopolamine treated group) without inducing any change in animal's memory *per se*. Moreover, it didn't alter mice locomotion. **Conclusion**: In summary, LFQM 75 can be considered a new lead compound for AD treatment. Additional experiments to further characterize its neuropharmacological profile are underway, including dose- response curves and other behavioral tasks such as Y maze and Morris watermaze. Financial Support: CNPq, FAPERJ, CAPES. All animal use protocols were approved by the Comission for the Ethical Use of Animals (CEUA-UFRJ, protocol number DFBCICB053).

13.010 Healing activity and anti-inflammatory action of the extracts from PE1, PE2, PE3 of the Amazon flora. Bastos AC, Santos GCQ, Gomes MF, da Silva JKR, Maia JGS, do Nascimento JLM, Bastos GNT UFPA

Introduction: Pressure ulcers (PR) are skin injuries located in the dorsal area where the pressure of boney prominence induce this disease by pressure or friction or bony in skin. This disease commits patients with low mobility that stay in the same position for long periods. **Aim**: Evaluate the anti-inflammatory and healing activity of three Amazon extracts of seeds in the pressure ulcers in vivo as a possible treatment. **Methods**: No definition, origin or description of extracts is show because those are involved in a process for obtaining a utility patent. The extracts PE1 (Baccharis sp), PE2 (*Pentaclethra sp*) and PE3 (*Omphalea sp*) which are rich in fatty acids, were extracted from the seeds of their respective plants, the seeds were pre-dried at 32ºC and dried at 38ºC, after 150 g of seed was milled, the milled seeds were mixed with 400 ml of acetone. After 72 hours it was filtered. The filtrate was kept at 4ºC in the refrigerator. All of them are largely used by local communities. The ulcer was induced by cycles of ischemia-reperfusion by applying and removing a rectangular permanent magnet in dorsal area of rat skin under which a ferromagnetic steel plate implanted. Twenty four male Wistar rats (6-8 weeks) were randomly allocated in six groups (four animals in each group): control (no treatment), PE1 (2.5mg in 0.5ml), PE2 (2.5mg in 0.5), PE3 (25mg in 0.5ml), diethylamine diclofenac® (0.04mg) and dersani® (2.5mg in 0.5ml), the concentrations above were used in each animal. The extract were used by topical and restrict to ulcer area. The lesion area measured by ImageJ software, exudates volume, number of migratory cells measure with Neubauer chamber and histological staining were investigated, to evaluate the effect of PE1, PE2 and PE3. All procedures involving animal care were performed in accordance with guidelines of the Ethical Committee for Research with Experimental Animals of the Federal University of Pará (123-13).**Results**: The ischemia-reperfusion promoted a lesion area of 87± 15.32%, while the lesion area of the ulcers treated with diethylamine diclofenac®, dersani®, PE1, PE2 and PE3 were significant decreased in 79±1.58%, 6.50±3.68%, 64.5±1.29%, 53.04±8.88%, 43.55±21.20% respectively. The volume of exudate in the control group was 4.25 ±1.25ml, while in the groups treated with diethylamine diclofenac®, dersani®, PE1, PE2 and PE3 the volumes were 1.37 ± 0.47mL; 1.68 ± 0.23mL; 1.7 ± 0.67mL; 2 ± 0.74mL; 1.4 ± 0,89mL respectively, thus showing a significant decrease in the volume of exudate. The number of migratory cells (Nºx106/mL) to the lesion area in the control group was 147,75 ± 39,79, while in the groups diethylamine diclofenac®, dersani®, PE1, PE2 and PE3 was 13.5 ± 9.14; 71.25 ± 12.33; 9.2 ± 6.78; 9.13 ± 7.79; 12.5 ± 9.96 respectively, showing an significant inhibition of the cellular migration to lesion area. The histological analysis to hematoxylin and eosin of the group treated with the PE1, PE2 and PE3 showed the best restructuring organization and skin structure when compared to positive and negative controls. **Conclusion**: These data suggest that the three extracts analyzed improved powerful healing activity in ulcers indicating a prominent alternative treatment for pressure ulcer. **Sources of research support**: **CNPq, CAPES, UFPA.**

13.011 Evaluation of plant extracts and synthetic compounds on secretion of insulin from langerhans islets. Iwamoto RD, Borck PC, Lubaczeuski C, Pereira CS, Sawaya ACHF, Landucci ECT, de Nucci G Unicamp – Farmacologia

Introduction: According to the International Diabetes Federation, there are 387 million people living with diabetes in the world (8.3% prevalence) and it is expected that the figure reach a staggering 592 million by 2035. Diabetes mellitus (DM) is a multifactorial disease characterized by chronic hyperglycemia due to the defect in insulin secretion by pancreatic β cell, insulin resistance or both. There are many classes of drugs available for glycemic control but the management of DM without or at least less side effects is still a challenge to the medical system. **Aim**: To evaluate the effect of 17 synthetic compounds and 3 plant extracts on secretion of insulin from Langerhans islets isolated from mice. **Methods**: All experimental procedures using animals were approved by Ethics Committee on Animal Use – CEUA/UNICAMP, number 3375-1. Langerhans islets were isolated from C57BL/6 mice by collagenase digestion protocols. The islets were incubated for 30 minutes at 37° C in a carbogenic atmosphere (pH 7.4). Then the Krebs solution was substituted by 1.0 mL of the same buffer containing different glucose concentrations: 2.8 and 22.2 mmol/L. The tested drugs were incubated for 1h with 5 islets per plate well and then the solution were collected for insulin dosage by radioimmunoassay. The aqueous extract of Mikania laevigata (AEML) were evaluated in Glucose Tolerance Test (GTT). After the first blood collection (basal), glucose was injected (1 g/kg weight, i.p.) and new samples were collected after 10, 15, 30, 60 and 120 minutes. Blood glucose level was determined by glucometer (Accu Check®, Roche, SP, Brazil) in tail vein blood and the area under the blood glucose curve was evaluated. **Results**: Synthetic compounds derived from pyridopyrimidines (50 µM) identified as 13 ($p < 0.05$), 16 ($p < 0.001$) and the AEML (5 mg, $p < 0.0001$) significantly increased the insulin secretion from Langerhans islets when compared to the control group (AUC). Further assay with AEML in the GTT showed a tendency to decrease the glycaemia according to the concentration of the extract solution given. **Conclusion**: Two synthetic compounds derived from pyridopyrimidines and the extract of Mikania laevigata increased the insulin secretion from Langerhans islets but further studies are needed to evaluate these substances (and isolated substances of AEML) on in vivo experimental protocols of pharmacology and toxicology. **References**: IDF. International Diabetes Federation Diabetes Atlas. Brussels, Belgium: International Diabetes Federation, 2013. Li, D. S., Y. H. Yuan, H. J. Tu, Q. L. Liang and L. J. Dai. A protocol for islet isolation from mouse pancreas. Nat Protoc 4(11): 1649-1652, 2009. Tabatabaei-Malazy, O., B. Larijani and M. Abdollahi. A systematic review of *in vitro* studies conducted on effect of herbal products on secretion of insulin from Langerhans islets. J Pharm Pharm Sci 15(3): 447-466, 2012.

13.012 Antibacterial activity and mechanism of hydroethanolic extract of *Gallesia integrifolia* **(Spreng.) Harms inner stem bark.** Karuppusamy A[1], Silva LI, Balogun SO, Martin DTO[2] [1]UFMT – Ciências da Saúde, [2]UFMT – Ciências Básicas em Saúde

Introduction: Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria. *Gallesia integrifolia* (Phytolaccaceae) is a shrub with glossy, elliptical leaves. It is widely distributed in the Atlantic Rain Forest of Brazil, occurring mainly in the states of Mato Grosso, Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo and Paraná. In traditional medicine, the decoction of the bark used for the treatment of infection, skin diseases, rheumatism, diarrhea, bronchitis, asthma, dermatitis, scabies, anaemia, stomach ache and cough by different place ethnic group peoples from Amazon basin. The aim of this study was to evaluate the antibacterial mechanism of *Gallesia integrifolia* (HEGi) plant extract against Gram-positive and negative bacterial species. **Methods**: HEGi antibacterial mechanism was studied by using following assays like; outer membrane permeability, nucleotide leakage, and efflux of K^+ ions. **Results and Conclusion**: In outer membrane permeability assay, erythromycin antibiotic exhibited more potent synergistic growth inhibition in association with ½ MIC of HEGi (MIC=400 µg/mL) against *Pseudomonas aeruginosa*. This result demonstrated that HEGi could increase the outer membrane permeabilization of *Pseudomonas aeruginosa* in association with erythromycin. The measurement of release of UV-absorbing materials is an index of cell lysis. After treatment with *Streptococcus pyogenes*, *Shigella flexneri* and *Staphylococcus aureus* resuspended in PBS, significant nucleotide leakage was observed after 6h of incubation, with the levels maintained through12h. In contrast, in the group containing *Shigella flexneri* (MIC=25 µg/mL) resulted in 83.8% nucleotide leakage occurred only at 12 h. Addition of HEGi (MIC=100 µg/mL) to a medium containing *Streptococcus pyogenes* caused two times increase in the leakage compared to that observed in the amoxicillin at 6h, peaking at12 h with a three times increase compared to the levels in the control. Addition of HEGi to a medium containing *Staphylococcus aureus* (MIC=400 µg/mL) resulted in a two times increase in leakage at 6h and a peak increase at 12 h compared to control. It is evident that the leak content depends on the incubation time. Bacterial cells treated with HEGi show a significant ($p < 0.001$) increase in leakage nucleotides in all the three bacterial strains. HEGi was induced immediate and massive effluxes of K^+. The increasing amount of potassium from *Shigella flexneri* and *S. typhimurium* after treatment indicate that HEGi may act on the plasma membrane by increasing permeabilization. These results supported that HEGi possess most effective against the Gram-positive and Gram-negative bacteria of outer membrane, disturbed the membrane, exhausted the intra cellular potential, and released cytoplasm macromolecules which lead to cell death. This plant extracts could be a potential source of new antibacterial agents. **Financial support and Acknowledgement**: CAPES, CNPq, INAU, FAPEMAT and UFMT.

13.013 Antitumor activity of the fractions containing three-finger toxins from the venom of the *Micrurus lemniscatus* (American Elapidic Snake): prospection of new molecules with specific pharmacology targets. Donato MF, Santos AK, Rios JPP, Batista-Filho FL, Pimenta AMC, Resende RR, de Lima ME UFMG – Bioquímica e Imunologia

Donato MF[1], Santos AK[1], Rios JPP[1], Batista-Filho FL[1], Pimenta AMC[1], Resende RR[1], de Lima ME[1] - [1]UFMG - Bioquímica e Imunologia **Aims**: Cancer is a serious public health problem in the world. According with the estimated by World Health Organization at 2012 were reported approximately 14 million new cases and 8.2 million cancer related deaths. Gliomas are the most frequent tumors occurring in the central nervous system (CNS) and are responsible for 80% of all malignancies of the brain and CNS. These tumors are categorized into four grades named I, II (astrocytoma, oligodendroglioma or oligoastrocytoma), III (anaplastic-astrocytoma/oligodendroglioma) and IV (glioblastoma). Compared with other types of tumors, gliomas are more challenging to treat because of the shield of the blood–brain barrier (BBB). In this context, new opportunities for efficient drug across the BBB are urgently needed. Many proteins and peptides possess biological those mark them as potential therapeutics or powerful pharmacological tools, in particular, for neurological disorders of the CNS. Snake venoms are complex mixtures of proteins, nucleotides and inorganic ions. The main toxins from *Micrurus* spp. venom are polypeptides neurotoxins, three-fingers toxins (MW= 6-8 KDa) and PLA_2 toxins with specifics activities in different pathways, mainly ionic channels and receptors. The goal of the present study was the prospection of the toxins isolated from *Micrurus lemniscatus* venom searching for antitumor activity on human gliomas, human neuroblastoma and human epidermoid carcinoma cell lines. **Methods**: Lyophilized crude venom of *Micrurus lemniscatus* was fractionated by RPC-HPLC. The isolated fractions were analyzed by MALDI-TOF mass spectrometry and used on the toxicity assays. Human glioma U87 (ATCC_HTB-14), U373, neuroblastoma SH-SY5Y (ATCC_2266), human epidermoid carcinoma A431NS (ATCC_2592) and HEK293 (ATCC_1573) control cells ($1x10^4$cells/well) were treated with different concentrations of toxins ($5x10^{-2}$, $5x10^{-1}$ and 5 µg/mL) at 24h. Cell survival was quantified by MTT method and viability assay for fluorescence. **Results**: Our preliminary results showed toxic effects of *M. lemniscatus* crude venom and its fractions on mice neuroblastoma cell line (Neuro-2A), after 24 h. Moreover, the crude venom (dose-dependent manner) and most of its fractions caused toxicity (40-80%) on cells. The F2, F9, F11, F22 and F24/25 fractions promoted significant cell death (60-80%) on Glioblastoma U87 cells (grade IV) and F3, F4, F24/25 and F26 caused toxicity (40-90%) on Glioblastoma-astrocytoma cells (grade III). However, the SH-SY5Y, A431 and HEK293 cell lines were not susceptible to the death. We observed that the isolated fractions tested and involved in cell death contained mainly toxins in the range of 6 to 8 kDa. **Conclusions**: These studies suggest a specific toxicity of some fractions to human glioma cells seems to be an evidence to bioprospecting of selective and promising antitumor drugs. **Suported by**: Fapemig; CAPES; INCTTOX; CNPq. Thank FUNED for providing the crude venom *of M.lemniscatus*.

13.014 Molecular dynamics study of Plasmepsin II inhibitors. Carlos E, Braz C, Guimarães E UFRN

Introduction: Malaria is still one of the biggest health problems worldwide; infections with malaria parasites result in one million victims annually. Plasmodium falciparum's, the most lethal malaria parasite, Plasmepsin II (Plm II) is a specific aspartic protease respçonsible for the hemoglobin methabolism. Molecular dynamic simulations have provided detailed information on the fluctuations and conformational changes of proteins and drugs. It was applied for the better undestanding of Plasmepsin biding site and consequently potential inhibitors bidinding mode. **Objectives**: Performing a simulation of the protein with their inhibitors, the biding mode could be studied. Hence the difficulty presented by the protein flexibility. **Methods**: Protein crystallographic form was taken from Protein Data Bank (PDB code : 4CKU) and was used to perform molecular dynamics along all the 54 chosen ligands retrieved from the literature. With GROMACS the protein structure was converted to a proper format using AMBER99SB-ILDN force field and the complex was embedded with implicit solvent. The system was submitted to geometry optimization using steepest descent and was submitted to a productive molecular dynamics simulation using 2000 ps using GROMACS package. **Results**: Dynamic binding phenomena was previously investigated with a chosen ligand and it was used to guide the remaining compounds whether the choice of the best conformations. It was observed that deviating from the crystal form the thiazolidinecarboxylic portion of the compounds occupied the protein S1' pocket and allowed the other part of the ligands to reach the S2' pocket. Reaching a more stable conformation than presented on the crystallographic form. Some compounds with lesses inhibitory activity appeared to have a different biding mode, interacting with the flap or not interacting at all with the S2' pocket of the protein. **Conclusions**: With a better understanding of the biding mode it was possible to reach better results when choosing the best conformation for the data set. This poses could be used to compose a predictive model of QSAR and give hints for structure-based antimalarial targets design.